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Colorimetric-based detection of *Ureaplasma urealyticum* using gold nanoparticles

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Abstract: *Ureaplasma urealyticum* (uu) is one of the most common agents of urogenital infections and is associated with complications such as infertility, spontaneous abortion and other sexually transmitted diseases. Here, a DNA sensor based on oligonucleotide target-specific gold nanoparticles (AuNPs) was developed, in which the dispersed and aggregated states of oligonucleotide-functionalised AuNPs were optimised for the colorimetric detection of a polymerase chain reaction (PCR) amplicon of *U. urealyticum* DNA. A non-cross-linking approach utilising a single Au-nanoprobe specific of the urease gene was utilised and the effect of a PCR product concentration gradient evaluated. Results from both visual and spectral analyses showed that target–Au-nanoprobe hybrids were stable against aggregation after adding the inducer. Furthermore, when a non-target PCR product was used, the peak position shifted and salt-induced aggregation occurred. The assay's limit of detection of the assay was 10 ng with a dynamic range of 10–60 ng. This procedure provides a rapid, facile and low-cost detection format, compared to methods currently used for the identification of *U. urealyticum*.

1 Introduction

Ureaplasma urealyticum (uu) is one of the most common human urogenital pathogens and mostly acquired via sexual contact [1]. It is a part of the normal vaginal flora with a worldwide colonisation value ranging from 60 to 80% [2]. Despite this, *U. urealyticum* has frequently been isolated in lower urogenital tract infections of both men and women [3]. Furthermore, several studies have implied the microorganism to be associated with additional pathological conditions such as: postoperative wound infections, chronic lung disease in neonates [4], non-gonococcal urethritis [5], prostatitis [6] and intrauterine infections including: endometritis, pelvic inflammatory disease [7], chorioamnionitis [8] and postpartum fever, leading to major complications such as preterm birth [9], spontaneous abortion, infertility [10] and perinatal mortality [11].

The genus *Ureaplasma* belongs to the family *Mycoplasmataceae* [12] and contains two common species, *U. parvum and U. urealyticum*. U. parvum is a commensal or low pathogenic species that is commonly found in healthy men and women, while *U. urealyticum* is a recognised pathogen that is less common [2]. The average global prevalence of this pathogen in people with infertility, adverse pregnancy outcomes and urinary tract infections is estimated to be 30–50% [13]. The significant clinical importance of this microorganism combined with its potential for asymptomatic genitourinary system colonisation makes its timely and sensitive diagnosis an urgent concern [14].

Currently, *U. urealyticum* is detected through two major strategies: bacterial culture-based phenotypic methods and nucleic acid detection assays. Culture is the gold standard for the diagnosis, but this organism is fastidious, requiring an intensely quality-controlled medium and several days of incubation; therefore, culture-based detection is costly and laborious [15]. In contrast, molecular-based methods like polymerase chain reaction (PCR) and real-time PCR using *Ureaplasma*-specific sequences provide a quick diagnosis with high sensitivity; and however, these methods are considered limited by the need for expert persons and by high equipment costs [16]. The distinct drawbacks of both approaches indicate a need for better detection solutions regarding this pathogen.

Lately, considerable interest has arisen in the utilisation of gold nanoparticles (AuNPs) for pathogen detection [17–20]. These assays mainly rely on the basic principles of surface plasmon resonance (SPR) of AuNPs to detect changes in nanoparticle aggregation state. AuNPs functionalised with thiol-modified oligonucleotides (Au-nanoprobes) possess unique optical properties and can be easily observed by the naked eye, with no requirement for complicated tools. These are used as signal transduction elements in the identification of nucleic sequences [21] using either cross-linking [22] or non-cross-linking (NCL) approaches [23]. In NCL colorimetric detection, the hybridisation of a specific complementary target to an Au-nanoprobe leads to electrosteric stabilisation of the AuNPs and resistance to salt-induced aggregation [21, 24]. This assay offers high sensitivity and specificity at much lower expense than with traditional methods [25].

The pronounced scattering intensity of AuNPs results from the collective oscillation of conduction electrons [26]. As the resonance band depends on AuNP size and shape [27], as well as the refractive index conditions in their close proximity [28, 29], it is possible to detect even single coupling events between functionalised nanoparticles [30]. Spherical AuNPs with an average size of 9–99 nm have been observed to absorb at 517–557 nm [31]. The AuNPs most easily synthesised, and therefore most commonly employed in biosensors, have sizes <60 nm and an absorbance peak at around 520 nm [32]. Monodisperse AuNP solutions have SPR peaks at almost 525 nm (red colour); in presence of salt, aggregate (blue colour) and the surface plasmon band is shifted to longer wavelengths (red shift) [33].

In the current study, we attempt to develop a colorimetric assay using AuNP-oligo probes for the first time to detection of *U. urealyticum*. This method uses the NCL approach and targets the urease gene of *U. urealyticum* using a specific probe.